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Fragile X Syndrome in Two Siblings With Major Congenital Malformations

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We report on 2 brothers with both fragile X and VACTERL-H syndrome. The first sibling, age 5, had bilateral cleft lip and palate, ventricular septal defect, and a hypoplastic thumb. The second sibling, age 2½, had a trachesophageal fistula, esophageal atresia, and vertebral abnormality. High-resolution chromosome analysis showed a 46, XY chromosome constitution in both siblings. By PCR and Southern blot analysis, the siblings were found to have large triplet repeat expansions in the fragile X gene (FMR 1) and both had methylation mosaicism. Enzyme kinetic studies of iduronate sulfatase demonstrated a two-fold increase in activity in the first sib as compared to the second. Possible mechanisms through which the fragile X mutation can cause down-regulation of adjacent loci are discussed.

KEY WORDS: fragile X syndrome, VACTERL-H syndrome, major congenital malformation, methylation mosaic, iduronate sulfatase

INTRODUCTION

Fragile X syndrome represents the most common form of inherited mental retardation. Phenotypic manifestations in childhood can be subtle and may only appear as prominent ears, developmental delay, and behavior problems [Hagerman and Silverman, 1991]. We report on 2 brothers with both fragile X and VACTERL-H syndrome. Molecular genetic and biochemical studies support the hypothesis that down-regulation of adjacent adjacent loci may be responsible for the occurrence of congenital malformations in these brothers.

CLINICAL SUMMARY

Sib 1

SR was born at 41 weeks of gestation by Caesarian section for breech presentation. Apgar scores were 5 and 9. Birth weight was 2.7 kg. Bilateral cleft lip and palate was evident at birth. A flexion contracture of the right thumb was also noted. Echocardiogram showed a small ventricular septal defect. Neonatal hypoglycemia and thrombocytopenia resolved. Psychomotor development was significantly delayed. Walking occurred at age 18 months. First words were spoken at age 2. An MRI scan of the brain was significant for mild ventriculomegaly. On physical examination at age 5⅓ years (Fig. 1), sib 1 had a height of 105.5 cm (10th centile), weight 16.5 kg (15th centile), and OFC of 49.7 cm (25th centile). The outer canthal distance was 7.9 cm (50th centile) and the inner canthal distance 2.7 cm (40th centile). The calculated interpupillary distance was 4.8 cm (40th centile). The frontal region was narrow. The palpebral fissures slanted upward. Mild synophrys and epicanthal folds were present. The ears were normally positioned and formed. The right and left pinna were 6.1 and 6.2 cm long, respectively (85th centile). The nasal bridge, tip, and left nasal alae were flattened. Bilateral cleft lip repair scars were evident. The anterior portion of the palate was not repaired. Bilateral clinodactyly of the fifth digit was present. The right thumb was hypoplastic. Genu valgum was present in both knees. Both testes were descended and measured 2 ml each (75th centile). No focal deficits were present on neurological assessment.

A psychological evaluation was done at age 4 years 8 months using the Merrill Palmer Scale of Mental Tests and the Vineland Adaptive Behavior Scales. A raw score of 36 was obtained, corresponding to a mental age of 34 months. These results fall within the range of

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Fig. 1. SR, age 5⅓ years. Bilateral cleft lip repair scars evident. Patient also has a small ventricular septal defect.

mental retardation. Expressive and receptive language skills were the most delayed. Adaptive functioning showed mild to moderate deficits, consistent with cognitive level. A psychiatric evaluation was significant for impulsiveness and distractability.

A developmental assessment performed at age 6⅝ years was significant for global delay, with most skills between 2 and 3 years below age expectancy. The child is currently enrolled in a special education program.

High-resolution chromosome analysis was significant for a 46, XY chromosome constitution.

Because of the history of neonatal thrombocytopenia and a thumb abnormality a DEB-induced chromosome breakage study was performed to rule out Fanconi anemia. This study showed no increased spontaneous or DEB-induced chromosome breakage rates.

Sib 2

MR was born at term and delivered by Caesarian section because of fetal distress. Apgar scores were 7 and 9. Neonatal respiratory distress and increased oral secretions prompted surgical evaluation. A tracheoesophageal fistula type C was diagnosed and repaired. A Nissen fundoplication was performed and a gastrostomy tube was placed. A T-4 butterfly vertebra was noted on chest film. Cardiac echo demonstrated the presence of a large secundum atrial septal defect located in the mid portion of the atrial septum. Renal ultrasound findings were normal. An MRI scan of the brain indicated mild dilatation of the lateral ventricles, temporal horns, and 3rd and 4th ventricles, consistent with mild to moderate communicating hydrocephalus. Psychomotor developmental was globally delayed. Standing occurred at 18 months and walking at 2.5 years. At the age of 3 the patient was nonverbal and displayed hand flapping, spinning, and rocking mannerisms.

Physical examination at age 2½ (Fig. 2) was significant for a height of 86 cm (15th centile), OFC of 48.3 cm (40th centile), and interpupillary distance of 4.8 cm (60th centile). Bilateral epicanthal folds were present. Both ears were prominent. The right and left pinna were 5.3 (60th centile) and 5.8 cm (85th centile) long, respectively. The nasal bridge was flattened and the palate was highly arched. Hyperextensibililty of the fingers and toes was observed. Testes were descended and measured 2 ml (75th centile). No focal deficits were present on neurologic assessment. High-resolution chromosome analysis showed a 46, XY, inv(9) (p11q13) chromosome complement.

With respect to their family history, both parents are Hispanic Americans who were born in Puerto Rico. The patient's mother has a brother who is reported to be "slow." The patient's father has a fourth cousin once removed with cleft lip and palate. Otherwise family history was unremarkable.

MOLECULAR AND BIOCHEMICAL FINDINGS

PCR analysis of the CGG repeat region of *FMR 1* [Brown et al., 1993] showed that both brothers had alleles of more than 200 repeats. Southern blot DNA analysis with probe StB12.3 showed that both were methylation mosaics (Fig. 3). The first had 300 CGG repeats with 75% of the DNA unmethylated, while the second had 600 CGG repeats with 25% of the DNA unmethylated. PCR analysis of the mother demonstrated two *FMR 1* alleles of approximately 31 and 75 CGG repeats, consistent with the carrier female pattern.

In order to determine if other loci on the X chromosome were inactivated in these sibs, enzyme kinetic studies of iduronate sulfatase (IDS) were performed

Fig. 2. MR, age 2½ years. Malformatons include a tracheoesophageal fistula, large secundum atrial septal defect, vertebral anomaly, and communicating hydrocephalus.

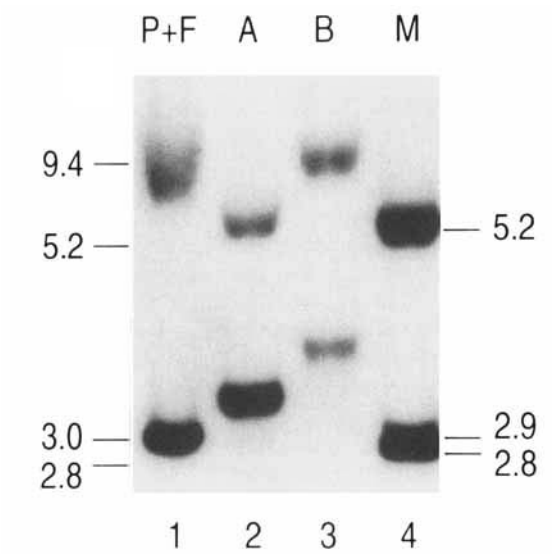

Fig. 3. Southern blot DNA analysis showed the 2 sibs were methylation mosaics. The method of Rousseau et al. [1991] was used and the blots show a double digest with restriction enzymes EcoR1 and Eag1. **Lane 1** was a mixture of DNA from a premutation male with 94 repeats and a full mutation male (P+F) in a ration of 44:56 premutation to full mutation used for calibration using a scanning densitometer. **Lane 2** was the first sib (A) with 64% of his DNA unmethylated. **Lane 3** was the second sib (B) with 42% of his DNA unmethylated. **Lane 4** was the mother of the two sibs with a doublet at approximately 2.8 and 2.9 kb. Molecular markers are shown on each side in kb.

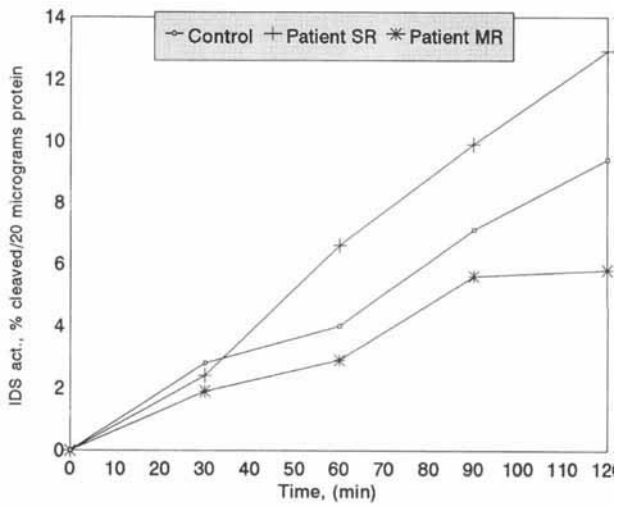

Fig. 4. IDS kinetic activity in WBC from the 2 affected sibs and a normal control. The IDS activity was determined in aliquots containing 20 μg protein, which was optimal for this assay. The incubations were carried out for the time periods indicated and the percent substrate cleaved was determined.

(Fig. 4). Substrate for IDS was prepared following degradation of heparin by butylnitrite. The disulfated discaccharide was isolated and then reduced with sodium borotritide to give the radioactive labeled substrate. IDS activity was measured in aliquots of whole blood cell homogenates following extensive dialysis as described by Hall et al. [1978]. Each incubation contained 50,000 CPM of substrate and 20 g of protein, which was considered optimal protein concentration. The enzymatic hydrolysis of the substrate was estimated as percent of the total radiocativity in the assay. Details of the method are outlined by Leder [1978].

Sib 1 was found to have a twofold increase in IDS activity (12.9% vs. 5.8%/20 g protein/2 hours) as compared to sib 2, which was within the normal range of 2.4 to 14.1% cleaved substrate observed in the coauthors' (R.M. and R.K.) laboratory. Sib 2 had consistently lower levels of IDS activity compared to sib 1 at all time points shown in Figure 4.

DISCUSSION

The fragile X phenotype in adults consists typically of prominent ears, prognathism, prominent forehead, high arched palate, and macroorchidism in association with mental retardation and behavior problems. Fragile X syndrome has been postulated to be associated with an underlying connective tissue dysplasia [Opitz et al, 1984; Hagerman, 1991], based on the observation of joint hypermobility, mitral valve prolapse, pes planus, and pectus excavatum in some affected individuals. Partington [1984] reported 5 of 61 male subjects with fragile X syndrome and cleft palate. He also described one patient with an atrial septal defect. Lachiewicz et al. [1991] described an association of Robin sequence with fragile X syndrome. A family with fragile X syndrome was reported by Loesch et al. [1992], in which some members had cleft lip and palate and other minor anomalies.

The incidence of fragile X syndrome in males has been estimated to be approximately 1 in 1,250 [Brown and Jenkins, 1992]. Cleft lip with or without cleft palate has an approximate incidence of 1 in 1,000 [Gorlin et al., 1971]. Tracheoesophageal fistula occurs with an incidence of 1 in 75,000 to 1 in 100,000 live births [Buyse, 1990]. The incidences of atrial and ventricular septal defects are approximately 1 in 1,000 and 1 in 400 respectively [Buyse, 1990]. Based on these frequencies, it seems unlikely that the presence of fragile X syndrome and the specific congenital malformations described in these brothers represent a chance event. To our knowledge these cases described here represent the first reported sibs with fragile X syndrome and multiple major malformations. These cases are instructive because they demonstrate the need to consider a diagnosis of fragile X in all children with delayed development, even when malformations are present.

Both sibs also fulfill the diagnostic criterion for VACTERL-H syndrome [Evans et al., 1989; Briard et al., 1984], which includes vertebral anomalies, anal atresia, cardiac anomalies, tracheoesophageal fistula, radial (limb), renal anomalies, and hydrocephalus. Although VACTERL-H syndrome usually represents a sporadic event, evidence for both autosomal recessive and X-linked recessive inheritance has been reported in several families [Sujansky and Leonard, 1983; Hunter and MacMurry, 1987; Wang et al., 1993]. Since both sibs have both fragile X and VACTERL-H syndrome, we

propose that the *FMR 1* locus may be closely linked to a locus for VACTERL-H. The presence of the fragile X mutation may alter gene expression at the neighboring VACTERL-H locus or a VACTERL-H contiguous gene segment.

The gene responsible for fragile X syndrome, *FMR 1*, can undergo a series of mutational events [Verkerk et al., 1991], including amplification of a trinucleotide (CGG) repeat and methylation of CpG dinucleotides leading to the full mutation. A CGG repeat increase of 60–200, corresponding to an increase by 90–510 bp is typically observed in carrier females and in transmitting males (premutation). Amplification of CGG repeats to the range of 200–1,300 (600–4,000 bp) is typically observed in females and males with a full mutation. This major amplification is usually associated with methylation of the upstream promotor, a CpG island, and transcriptional inactivation of *FMR 1* gene expression. Amplification of CGG repeats in the range of 600–4,000 bp has been observed in affected males and females with hypermethylation resulting in transcriptional inactivation of *FMR 1* [Pieretti et al., 1991]. Some affected individuals display somatic instability of CGG repeat segment length in peripheral blood samples [Nolin et al., 1994]. These individuals display a range of DNA fragment sizes and methylation patterns which may include both the premutation and full mutation. The mosaic pattern can reflect both methylation and size variations [Nolin et al., 1994].

In order to determine whether *FMR 1* methylation results in inactivation of adjacent loci, Clarke et al. [1992] studied the effect of *FMR 1* methylation on IDS activity, since the IDS gene was the closest known gene located 1 mb distal to *FMR 1* . They observed that fragile X males had significantly lower levels of serum IDS as compared to control males. Although no difference in methylation patterns between fragile X and control samples at the IDS locus were observed, the IDS probe used did not contain any genomic sequences 5' to the IDS coding region. It is possible that the relevant methylation changes were not identified.

Alternatively, it is possible that the mechanism by which loci adjacent to the *FMR 1* are down-regulated does not involve methylation. *FMR 1* codes for a protein (FMRP), which is a cytosolic RNA binding protein and interacts with a subset of brain mRNA [Siomi et al., 1993; Ashley et al., 1993; Gibson et al., 1993]. It has been suggested that FMRP could control the translation of selected mRNAs within the neuron. Decreased translation of these mRNAs could then account for the phenotypic features observed in fragile X syndrome. Although not all individuals with fragile X syndrome have congenital malformations, it is possible that the absence of FMRP in some individuals may further decrease translation of mRNAs which code for proteins expressed in other tissues besides brain.

Both sibs displayed a mosaic *FMR 1* methylation pattern. SR had 300 trinucleotide repeats, of which 75% of *FMR 1* DNA was unmethylated by Southern blot analysis. Although both sibs have the same genotype with respect to the X chromosome, SR had consistently higher IDS levels at all time points shown in Figure 4. His serum IDS was twice as high as that of MR, who had 600 trinucleotide repeats with 25% *FMR* 1 unmethylated. These data support the observations of Clarke et al. [1992], and suggest that the observed variation in IDS levels is influenced by the level of methylation and/or CGG repeat number in the *FMR-1* gene.

The significance of mosaicism on development is unclear. In one family studied by McConkie-Rosell et al. [1993], an inverse correlation was observed between the degree of methylation and cognitive level. It is also possible that somatic instability as reflected by the mosaic methylation pattern may be responsible for the discordant phenotype of these sibs.

In summary, these two male sibs with fragile X syndrome had methylation mosaicism and multiple congenital malformations consistent with the VACTERL-H syndrome. We postulate that inactivation of other loci adjacent to *FMR 1* is responsible for the occurrence of multiple major congenital malformations in these sibs.

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